THE HUMAN FECAL STREPTOCOCCI

C. J. Oppenheim

From the Department of Hygiene and Bacteriology, University of Chicago

Ever since the discovery of the typhoid bacillus by Eberth in 1880, gastro-intestinal bacteriology has concerned itself largely with the study of various organisms that have been thought to have a possible bearing on the etiology of specific diseases. It is not surprising, therefore, that among the large number of organisms isolated from the intestinal tract and studied, the streptococcus has come in for its share of attention as a possible inciter of certain of the diarrheal diseases as well as local gastro-intestinal disturbances in infants and adults.

As early as 1890, Kruse and Pasquale[1] in an investigation on dysentery and tropical abscess, found streptococci to be the predominant organism in dysentery stools, and though they observed that streptococci also seemed to predominate in the diarrheal stools of nondysenteric origin, they nevertheless felt the possibility of etiologic relationship between this disease and streptococci. Beck[2] reported a case of cholera nostras in which he was able to isolate "almost pure cultures" from the stools on the third day of the disease. Streptococci isolated from the organs of the case were found to be highly pathogenic for mice. He concludes that the streptococci isolated were the causative organisms.

Metschnikoff[3] mentions Drasch's report of a streptococcus which, after isolation from a case, was found to be able to produce diarrheas resembling in all respects those provoked by the cholera vibrio. Askanazy[4] reports a case of phlegmonous enteritis with fatal termination in which streptococci seemed to have had some provocative significance. A series of small epidemics of severe gastro-enteritis, apparently due to the consumption of raw milk in Christiania, were studied by Axel Holst[5] in 1895 and the blame placed on a cow found to harbor streptococci in a subacute udder infection. The streptococci isolated were able to produce a rather intense hyperemia of the intestinal vessels of mice and dogs to which cultures were fed, and provoked diarrheal attacks when cultures were swallowed by Holst and his colleagues. Holst remarks on the variance in virulence of these organisms, which seemed to be morphologically and culturally identical with the streptococci ordinarily found in the Christiania market milk.

Received for publication Oct. 4, 1919.

1 Ztschr. f. Hyg. u. Infektionskr., 1891, 16, p. 1.
2 Centralbl. f. Bakteriol., 1892, 12, p. 632.
3 Ann. de l'Inst. Pasteur, 8, p. 258.
4 Centralbl. f. allg. Path. u. path. Anat., 1895, 6, p. 313.
5 Baumgartens Jahresbericht, 1896, 52, p. 545.

Tavel, Cérenville, Eguet, and Krumbein[6] noted a series of cases of severe gastro-enteritis of a somewhat typhoidal clinical course, in which a peculiarly varying streptococcus was found. Cérenville believed this organism differed perceptibly in morphology from the normal intestinal streptococci and termed the clinical cases "streptococcus enteritis."

Lameris and Harrevelt[7] record an epidemic in a hospital in which boiled milk, previously heavily polluted from the milk of a cow recovering from a subacute mastitis, was being used; and attributed the epidemic to toxic material produced by the organisms in the milk, though live cultures were found to be nonpathogenic for laboratory animals. Baermann and Eckersdorff[8] studied a series of cases of dysenterial appearance occurring in a Sumatra hospital in which only hemolytic streptococci were to be found in the stools. These organisms failed to have sharply defined agglutinative powers with the patients' serum, and produced no changes in macacus monkeys to which broth cultures were fed.

Ungermann,[9] in a bacteriologic investigation of appendicitis, found streptococci in 47% of 38 cases of acute and chronic appendicitis. He found, however, that staphylococci occurred in about 75% of the cultures from the same series. In 1916, Rosenow and Dunlap[10] reported an outbreak of appendicitis at Culver Military Academy, which they attributed to streptococci probably disseminated in dairy products. These streptococci, in a number of cases, produced in animals lesions resembling appendicitis.

As early as 1886, streptococci were recognized as normal inhabitants of the bowel of infants by Escherisch,[11] who described their morphology in detail. Czerny and Moser[12] described their appearance in blood cultures from cases of infantile gastro-enteritis. Fischl[13] studied 22 cases of septic gastro-intestinal conditions in sucklings, and found streptococci to be the predominating organisms.

In 1895, Booker[14] made a thorough study of over 100 cases of summer diarrhea, covering a period of 5 years' observations, in which bacteriologic and anatomic findings were correlated. He concluded that summer diarrhea, though not attributable to any single micro-organism, may be induced by the activity of several varieties of ordinary occurrence and wide distribution, the principal ones being B. proteus and the streptococcus.

Several cases of so-called "streptococcus enteritis" in sucklings were reported by Hirsch,[15] Libman,[16] and Spiegelberg[17] from the clinic at Graz, and were reviewed by Escherisch.[18] The interesting feature of these cases is the fact that practically all occurred in infants reared on cow's milk, and that streptococci predominated in the blood cultures and were found in large numbers in bacteriologic examinations of the stools.

[6] Ann. Suisses des Sc. Méd., Ser. H, 2, 11.
[7] Ztschr. f. Fleisch u. Milch Hyg., 1901, 11, p. 114.
[8] München. med. Wchnschr., 1909, 56, p. 1169.
[9] Centralbl. f. Bakteriol., I, O., 1909, 1, p. 513.
[10] J. Infect. Dis., 1916, 18, p. 383.
[11] Die Darmbakterien des Saüglings, 1886.
[12] Jahr. f. Kinderh., 1894, 38, p. 430.
[13] Ztschr. f. Heilk., 1894, 15.
[14] Johns Hopkins Hosp. Repts., 1897, 6, p. 159.
[15] Centralbl. f. Bakteriol., 1897, 22, p. 369.
[16] Ibid., p. 376.
[17] Ibid., 1898, 24, p. 401.
[18] Jahrb. f. Kinderh., 49, p. 1898.

Jehle[19] presented a study of 49 cases of enteritis in sucklings and concluded that the evidence points to a milk-borne streptococcus infection. The impression seems to be general that cow's milk offers more opportunity for infection with streptococci than human milk, and the tendency is rather to underestimate other factors incident to artificial feeding which might profoundly influence the opportunity for, and the unfavorable outcome of, infection. In this connection, it is interesting to find that Cohn and Neumann[20] have found that human milk commonly contains pyogenic cocci, including streptococci.

The difficulty of evaluating the literature of streptococcal alimentary infection is at once apparent, since the allusions are rather fragmentary and for the most part based on the report of single or small series of cases, in which the bacteriologic investigations have usually been carried out under older methods or with insufficient detail. These frequent allusions have also, as Nobécourt[21] has observed, tended rather to complicate than to simplify matters, since the streptococcus has long received recognition as a normal inhabitant of the skin and mucous membranes; and since morphologic, biologic and pathogenic characteristics have not assisted materially in bringing about a differentiation of the pathogenic from the nonpathogenic strains. Newer methods of classification based on a combination of observations on growth on blood mediums, as originally proposed by Schottmüller.[22] and fermentative groupings originating with Gordon,[23] have perhaps given a little more hopeful aspect to the possibility of identifying certain types as normal flora, and in addition have brought about the necessity of restudying these organisms under more modern methods.

The first exhaustive studies on the human fecal streptococci were made by Houston[24] in 1904, who examined 300 strains taken from 19 stools, and classified them on the basis of the Gordon fermentations. Houston found that the organisms fall into about 40 groups in their fermentative characteristics, and proposed 10 large classes into which the majority of his strains fell. In a later work, Houston reported on an additional 100 strains.[25]

Winslow and Palmer[26] reported on the fermentative characteristics of 116 strains of streptococci isolated from 15 samples taken from 10 persons, the majority of which were obtained from diarrheal stools. Fuller and Armstrong[27] made further studies on fecal streptococci, and reported on 123 strains, using methods of study similar to those employed by Winslow and Palmer.

19 Jahrb. f. Kinderh., 1907, 65, p. 40.
20 Virchow's Arch., 1891, 126, p. 391.
21 Presse méd., 1903, 2, p. 677.
22 München. med. Wchnschr., 1903, 1, p. 849.
23 Report of the Medical Officer, Local Gov't Board, 1903-4.
24 Ibid., Supplement, 1904-5.
25 Fifth Research Report, Metropolitan Water Board, 1910.
26 J. Infect. Dis., 1910, 7, p. 1.
27 Ibid., 1913, 13, p. 442.

Aside from these observations, there exist practically no studies on the streptococci of normal feces with the exception of strains which Broadhurst[28] has included in her studies, and 53 strains reported by Holman.[29] With the exception of the last two, no account has been taken of the characteristics on blood mediums. One thing, and practically the only one on which all observers seem to agree, is the rather constant presence of mannite-fermenting organisms in human feces, thus giving some justification to the name "fecalis" which has been used in all of the later classifications from that of Andrewes and Horder[30] up through the more recent groupings suggested by Blake[31] and by Holman.[29] Numerical estimations of the preponderance of mannite-fermenters in human feces are, however, at great variance. Thus Houston reports 24%, Winslow and Palmer 28%, Broadhurst 45%, Holman 50%, and Fuller and Armstrong 65%.

On closer examination, it is apparent, however, that the dissimilarity is not so pronounced as one would be led to believe by a mere casual survey of the percentage tabulations. The number of persons examined has been variable, and is not mentioned by some of the observers. The number of strains from each sample also varied greatly; and the methods of study were so different that it would scarcely be expected to find much greater accord in the results. Certain of these points will be taken into consideration later in this paper in more detail.

A conservative point of view is highly desirable in the interpretation of any infection along the basis of streptococcal specificity; and though the limitations of present methods of study of the streptococcus group are perhaps great, there nevertheless exists the desirability of establishing a norm in accord with the recent methods of classification, deviations from which may aid us in more firmly establishing future implications of streptococcal specificity. Up to the present, most observers have rather casually taken "streptococcus enteritis" and other "streptococcal" alimentary infections for granted. The case against the streptococcus rests, however, on a very insecure footing and will remain so until more comprehensive and precise studies of the normal streptococcal flora of the alimentary tract have been made.

The present study represents the examination of 55 stools from 15 persons, from which 323 strains were isolated. All the subjects were healthy adults, and care was taken to avoid any material which was of diarrheal character. The distribution of the samples and strains was:

[28] Ibid., 1915, 17, p. 277.
[29] J. Med. Research, 1916, 34, p. 377.
[30] Lancet, 1906, 2, p. 708.
[31] J. Med. Research, 1917, 36, p. 99.

TABLE 1

DISTRIBUTION OF SAMPLES AND STRAINS FROM PERSONS EXAMINED

Subject	Samples	No. of Strains
1	5	49
2	5	33
3	2	16
4	2	14
5	4	20
6	4	20
7	4	17
8	4	21
9	4	20
10	4	20
11	4	21
12	4	20
13	4	21
14	4	20
15	1	11

METHODS

Material.—Fecal material was collected in sterile paraffined pasteboard containers. The stools were inspected to eliminate loose, watery stools which occasionally were found incident to slight transient diarrheas in the subjects under observation. Material was plated as quickly as it could be obtained, and in no case did more than four hours elapse between deposition of the stool and plating. Plates were always made immediately before use, as it was found that the organisms grew more readily on fresh, moist blood agar.

A few loops of feces taken from the center of the stool were placed in tubes containing sterile salt solution, and after shaking until a uniform, slightly turbid suspension was obtained, two or three loops of the suspension were placed on the edge of a fresh plate. The loop, after being resterilized in the flame, was used to spread the suspension across the plate in radial streaks, "fan-wise" with cross streaks at the spread ends.

Plates were incubated for 18-24 hours at 37 C., at the end of which time, colonies appearing to be streptococci were fished into glucose broth and re-incubated. Replatings were then made from broth, and after the usual incubation, a single colony was picked and streaked on a fresh blood-agar slant. Such slants were used as stock slants, and all morphologic study and transfers to carbohydrate mediums were made from them.

Morphologic study included a Gram stain, and examination for capsule by means of the Welch capsule stain and hanging drop with darkened field.

The appearance of colonies was noted from the replating; transfers to carbohydrate mediums were made from slants, and readings made at the end of 5 days' incubation. All negative fermentations were controlled by three methods: (1) Morphology, (2) re-inoculation into glucose broth to demonstrate viability, (3) retesting in the carbohydrate which failed to show signs of fermentation. Fermentations were in this way controlled to assure the presence of growing organisms in the mediums. Practically all negative fermentations have been repeated twice, and in every case at least once, before recording.

Mediums.—Meat infusion has been used entirely for the base of carbohydrate mediums in which fermentative characteristics were studied, and also in the preparation of agar. The broth has been rendered "sugar-free" by incubating for about eight hours at 37 C. with heavy inoculations of B. coli. Two per cent. of peptone (Armour's) and ½% of salt have been used. The various carbo-

hydrates were added to the quantity of 1% (except in the case of salicin, when ½% was employed) and were added directly to the broth. All broths were titrated to approximate neutrality to phenolphthalein (hot). Determination of the hydrogen-ion concentration of several lots of broth showed a quite uniform P_H value of 7.5 or slightly higher (more alkaline).

One and a half per cent. agar prepared from the meat infusion was found satisfactory for both blood-plates and stock slants. Greater amounts of agar tend to decrease the moisture of the plates and slants, and thereby impair both the growth and longevity of the organisms. Green formation was found to occur most readily on a 10% blood-agar made from sterile defibrinated sheep blood. The formation of green pigment seems to vary greatly and be favored by the alkalinity of the plate. It was found that a P_H value of about 7.5 forms an excellent base for the demonstration of methemoglobin on the blood-plate.

Sterilization.—All mediums have been sterilized by autoclaving for 10 minutes at 10 lbs. of pressure. This was found fully satisfactory after comparison with fermentations in carbohydrate mediums sterilized by the fractional method.

Indicator.—In recent years, biometric studies with titration methods have frequently been used in the study of fermentations. Such methods are not without certain disadvantages: 1. They are more cumbersome and time-consuming. 2. They allow for an element of personal error which perhaps exceeds methods in which a sharply defining two-color indicator is employed. 3. They tend to give a fictitious security in quantitative values, which are by no means uniform, especially where different mediums are employed. This has been thoroughly studied, with regard to nutrient qualities, by Broadhurst;[32] and by Bronfenbrenner and Schlesinger[33] with reference to peptone, buffer and carbohydrate content. 4. Even in biometric studies, an arbitrary point has to be agreed on as the criterion of fermentation, so that the method does not therefore excel the use of any well chosen indicator. I have, therefore, used an indicator without titration in the routine study of my cultures. In the selection of an indicator, it is necessary to consider the initial reaction of the mediums, the range of acidities characteristically produced by the species of organism being studied, and the range at which the indicator employed customarily undergoes its color change.

"Bromcresol purple" (dibromo-ortho-cresol-sulphone-phthalein) was used, as suggested by Clark and Lubs[34] for milk, except that only half the quantity was found necessary to use in broth (5 c c to the liter of broth). These authors[35] denote the useful range of this indicator as lying between P_H values of 5.2 and 6.8, which is perhaps preferable for the demonstration of acidity in a medium of as distinct alkalinity as that which I have employed, and fully coincides with the amount of change in hydrogen-ion concentration which I have been able to demonstrate in 5-day incubations of several of my strains (vide infra).

In addition, this indicator has the advantage of sharp color contrast (from purple to bright yellow) on acidification, and is not reduced or impaired by autoclaving. (The "CR" indicator proposed by Bronfenbrenner[36] because it is much less expensive, would perhaps serve the purpose as well, since its range of usefulness about corresponds to that of bromcresol purple.)

[32] J. Infect. Dis., 1913, 13, p. 404.
[33] Proc. Soc. Exper. Biol. and Med., 1918, 16, p. 44.
[34] J. Agric. Research, 1917, 10, p. 105.
[35] J. Bacteriol., 1917, 2, p. 104.
[36] J. Med. Research, 1918-19, 49, p. 25.

OBSERVATIONS

Appearance of Colonies.—On blood-agar plates, the characteristic colony is the small, granularly opaque, varying from about one to two millimeters in diameter. With the plate on a white background, it is rather easy to differentiate the streptococcus colony by the slightly darkened zone about it; by transmitted light, a distinct green discoloration is readily noted about the colony in a medium of proper chemical reactivity.

I have been unable to distinguish the so-called "indifferent" variety of streptococcus among those isolated from feces. At times, colonies have failed to produce green on blood-agar, but no strain has persistently maintained indifference to the formation of green. It would seem highly suggestive that certain qualities in the medium are the principal determinants of methemoglobin formation. I have found agar of rather pronounced alkalinity (P_H of 7.5 or higher) to favor the production of green pigmentation.

Hemolytic streptococci in the feces of man are apparently the exception and not the rule. Davis[37] in an examination of 53 stools from a series of persons, several of whom were known to harbor hemolytic streptococci in their throats, failed to find a single hemolytic colony. On the other hand, Holman, in his studies reports the finding of 9 hemolytic strains out of a total of 53. Similarly, Broadhurst reports 9 hemolytics in her series of 31 fecal streptococci. I have been able to recover hemolytic streptococci from 5 persons in 15, twice from 2 and once from 3. They were apparently transient or accidental in occurrence, but their presence was nevertheless noted. As to constancy, the 12 strains isolated remained hemolytic over a period of months without exception. No attempt was made to estimate the percentage of hemolytics in stools where they were noted, but a rather general distribution usually occurred, though in no case did they predominate. In the two persons in whom they occurred twice, they appeared in the first and fifth, and first and third samples examined, the stools being collected at about weekly intervals.

General Morphology.—All organisms studied were gram-positive. The appearance of a capsule was rarely noted. One strain (239) maintained a capsule through several transfers. This strain also fermented inulin persistently. Insolubility in bile, however, caused me to disregard the possibility of its being a pneumococcus.

The typical morphology of young cultures in plain broth or from agar slants, was that of a short-chained, diplococcoid, rather lanceolate and coarsely beaded streptococcus. Earlier observers were inclined to confer a certain characteristic morphology on streptococci from various sources. A great number of bacteriologic studies on streptococci isolated from normal and pathologic fecal material from both infants and adults are given over to the description of the organisms. The value of such observations is perhaps quite slight, since it is possible to alter the appearance quite freely with conditions of growth. Thus, in glucose broth after 18 hours' incubation, the majority of fecal organisms tend to become diplococcoid, even in chain formation rather lanceolate and swollen. Addition of calcium carbonate to the glucose broth, which provides for a permanent alkalinity, allows the organisms to become smaller and rounder, and grow out in long strands. Morphology, therefore, would seem to vary with luxuriance of growth and reaction of the mediums.

[37] J. Am. Med. Assn., 1919, 72, p. 319.

Fecal streptococci are, in general, rapidly growing, active organisms. From this standpoint there might be some basis for characterizing them as occurring in short chains and with a tendency to diplococcoid appearance. Over 100 strains were tested for bile solubility. I was unable to find any bile soluble strain in my series.

Constancy of Fermentative Characteristics.—In general, there has been but slight, almost insignificant variation in the constancy of fermentative characteristics. Walker[38] believes that inconstancy is due to transient changes in the metabolic reactions of the organisms. Thro[39] has shown that repeated replatings give rise to single colonies which vary in their fermentative characters. Broadhurst[28] noted two varieties of inconstancy; one dependent on physical or chemical factors which tends to be rather inhibitory and temporary; the other due to marked environmental changes and tending to awaken more active, latent fermentative powers which remain more fixed and permanent.

Too much emphasis cannot be placed on carefully avoiding the acquisition of a mixed culture. In these studies, the progeny of a single colony from a subculture has been selected for study. Other factors that enter prominently into errors which may be interpreted as variability in reaction are the use of mediums which are not uniformly prepared. Thus Broadhurst[32] has found wide variation in acid production by identical strains in meat extract and meat infusion. Titration studies of 10 of my strains (314 to 323) made after 5 days' incubation at 37 C., of mannite and lactose-broth cultures in measured amounts of the extract and infusion, showed markedly higher acid production throughout the infusion cultures in spite of the fact that both extract and infusion cultures were proved to be viable at the end of that time (See table 3, p. 126).

The use of carefully controlled fermentations helps to eliminate the report of a negative fermentation where a failure to inoculate or to grow has accidently occurred. Variability after animal passage is always susceptible of the error inherent in the use of laboratory animals, namely, failure to recover the same organism that was introduced into the animal. This is particularly true of streptococci, which are commonly found as secondary and terminal invaders of the blood stream. Of especial interest in this connection are the observations of Richey and Goehring,[40] who were able to obtain positive postmortem blood cultures in about 33% of 206 cases within 10 minutes after death, and in a large number of antemortem results taken from the arm vein immediately before death; and who found cocci to be the most frequent invaders.

Nevertheless, it must be admitted that the nonhemolytic group of streptococci present a more heterogeneous complex than the hemolytic group, as is readily seen in the lack of specificity of complement fixation phenomena noted by Kinsella and Swift.[41] The general tendency, however, is for the large majority of streptococci under normal conditions of growth and preservation in vitro, to retain rather fixed fermentative characters. It is, then, unlikely that slight variations frequently observed will be sufficient to vitiate the value of fermentative differentiation.

[38] J. Path. and Bacteriol., 1912-13, 17, p. 140.
[39] J. Infect. Dis., 1914, 15, p. 234; 1915, 17, p. 227.
[40] J. Med. Research, 1918, 38, p. 421.
[41] J. Exper. Med., 1917, 65, p. 40.

Fermentations.—Early studies on the streptococci of normal human feces have usually embraced a grouping based on all or several of the Gordon test mediums. In recent years, the tendency has been to reduce the number of test substances, and in this way to avoid "species-mongering." In these studies, for the most part, all fermentative differentiation has been attempted on the basis of fermentations in glucose, lactose, mannite and salicin, so that together with the blood-plate findings, the organisms may readily be identified in the new groupings suggested by Blake[31] and Holman.[29]

All organisms fermented glucose. One strain (14) was markedly less vigorous in its fermentation of this carbohydrate than the others, which rapidly changed the color of the indicator. Of 323, but 8 failed to ferment lactose; 9 strains failed to ferment salicin; 84 failed to ferment mannite. All strains but 2 were negative in inulin broth. About 80 strains were tested in raffinose broth, and but 3 fermented this carbohydrate. A classification of the nonhemolytic streptococci found in human feces, based on a gross percentage from my studies, and classified according to the groupings of Blake and of Holman is:

Blake =	Str. fecalis	74.0%
	Str. buccalis	24.5%
	Str. equinus	1.5%
Holman =	Str. fecalis	73.0%
	Str. salivarius	1.0%
	Str. nonhemolyticus i	23.0%
	Str. equinus	1.5%
	Str. ignavus	1.5%

The result of these studies apparently would justify the assumption that the predominant fecal streptococcus is a nonl emolytic, mannite fermenting organism. That mannite fermenting organisms are characteristic of human fecal streptococci is a generally acknowledged fact, but their numerical frequency according to the results of various observers is somewhat in question.

In the following table are arranged the percentage fermentations in several carbohydrates, noted by various observers, together with my own observations:

TABLE 2

PERCENTAGE OF FERMENTATIONS IN VARIOUS CARBOHYDRATES

Observer	Glucose	Lactose	Salicin	Inulin	Mannite
Houston	..	76	93	5	24
Winslow and Palmer	89	62	..	..	28
Fuller and Armstrong	95	94	..	..	65
Broadhurst	..	87	96	16	45
Holman	..	100	99	2	50
Oppenheim	100	97	97	1	76

At once, it is apparent that there exist many points in common between the data of these observers; but it is equally true that there are many things on which the numerical evidence seems to be quite variable.

In considering the work, it is necessary to survey the several methods used by the workers in obtaining their results. Houston's fermentations were noted in meat extract broth after 48 hours' incubation, with litmus as an indicator.

Winslow and Palmer's fermentations were noted by titration methods in meat extract broth after 3 days' incubation; Fuller and Armstrong used practically the same methods as Winslow; Broadhurst used titration methods, but with meat infusion; Holman employed longer incubations in serum broth and used Andrade's indicator; while in my studies, 5-day incubations were read in meat infusion broth that had a distinct initial alkalinity, with bromcresol purple as an indicator.

Variations in mediums alone suffice to allow large differences to occur. Thus, 10 strains inoculated into 5 c c quantities of meat extract and meat infusion broths and titrated at the end of 5-days' incubation, gave these differences: All strains titrated were retransferred to glucose broth, in which they grew well, proving viability. It is obvious from the tabulations that meat infusion is a better medium for growth and for the demonstration of acidity production than is broth made from the extract.

TABLE 3

Differences in Results with Various Mediums

Strain	Lactose		Mannite	
	Extract	Infusion	Extract	Infusion
314	1.1	3.1	0.1	0.2
315	1.4	3.2	0.2	0.5
316	1.6	3.0	1.0	2.6
317	1.4	2.9	1.0	2.8
318	1.2	3.2	0.8	2.4
319	1.3	3.0	0.6	2.5
320	1.1	3.1	0.8	2.7
321	1.1	3.1	0.8	2.7
322	0.8	3.0	0.6	2.3
323	0.8	3.0	0.7	2.2

Initial p_H of infusion broths = 7.3; of extracts = 7.1.

The element of time also affects the readings greatly. It is therefore important in comparing fermentation readings, to ascertain whether readings were made on the second, third, fourth or fifth day. Frequently mannite fermentation was not complete until the fourth day, so that readings taken prior to this would have led to negative recordings. The choice of indicator and the range at which it best operates has been mentioned. Control of negative fermentations by repetition also helps in eliminating observational errors.

An examination of the changes in final hydrogen-ion concentration produced in 5 c c quantities of mannite and lactose broths, by several strains picked indiscriminately from my stock cultures and inoculated from newly incubated 24-hour blood-agar slants proves interesting (Table 4).

A noteworthy point illustrated by the tabulations is that, although lactose-broth changes are quite uniform, the changes in mannite are more variable and less sharply demarcated; and in several instances did not correspond with the classification of the strains as made by the routine methods with an indicator in these studies; this only by way of further suggesting the differences inherent to a fermentative classification.

It remains to be said, however, that mannite fermentation seems to be the crucial characterizing feature of a large part of fecal strains among the non-

hemolytic streptococci; this has been accorded recognition by most workers on classification, and by the few who have studied the human fecal streptococci, and suggests the possibilities of an as yet unworked practical application.

TABLE 4

FINAL HYDROGEN-ION CONCENTRATION CHANGES IN MANNITE AND LACTOSE INFUSIONS

Strain		Type (Blake)	Change in p_H Concentration in Mannite	Change in p_H Concentration in Lactose
1	25	Fecalis	2.0	2.6
2	120	Buccalis	0.8	2.6
3	47	Fecalis	2.2	2.8
4	41	Buccalis	2.2	2.7
5	13	Buccalis	0.0	2.7
6	50	Buccalis	0.0	2.7
7	52	Buccalis	0.0	2.4
8	110	Buccalis	0.0	2.7
9	66	Buccalis	0.0	2.6
10	74	Buccalis	0.0	2.4
11	67	Buccalis	0.0	2.6
12	82	Buccalis	0.0	2.6
13	152	Fecalis	2.5	2.4
14	33	Fecalis	2.5	2.5
15	135	Fecalis	2.4	2.6
16	95	Buccalis	0.0	2.3
17	73	Buccalis	0.0	2.6
18	118	Fecalis	2.5	2.7
19	177	Fecalis	2.6	2.5
20	77	Buccalis	0.0	2.6
21	233	Buccalis	0.1	2.6
22	46	Buccalis	0.1	2.7
23	189	Fecalis	2.4	2.6
24	133	Fecalis	2.3	2.7
25	104	Fecalis	2.5	2.6
26	248	Buccalis	1.3	2.3
27	100	Buccalis	0.0	2.6
28	194	Fecalis	2.5	2.5
29	274	Fecalis	1.8	2.5
30	116	Fecalis	2.4	2.7
31	151	Fecalis	2.4	2.7
32	56	Fecalis	1.6	2.4
33	273	Buccalis	2.0	2.6
34	72	Buccalis	1.6	2.3
35	2	Fecalis	2.2	2.5

Average p_H of sterile incubated broths = 7.3.
These determinations were made with colorimetric methods proposed by Haskins.[42] I am indebted to Miss Helen Penn for valuable assistance in making the determinations.

In the studies of Winslow and Palmer, 85 of 116 strains of the organisms isolated were taken from diarrheal stools; and several strains (36) failed to produce acidity in lactose, a few (9) even proving negative in glucose. This has suggested the possibility that some variation may exist in the fermenting power of streptococci of diarrheal stools.

With this in mind, I have examined 4 stools from typhoid patients, of distinctly diarrheal character; 2 from persons suffering from diarrheas of nonspecific origin; and 2 from a patient after free catharsis with a nonantiseptic purgative (elaterin). The organisms isolated were studied in the same manner as those from normal stools, and the same general appearance of colonies and morphology noted. A total of 84 strains was studied, no hemolytics occurring.

The tabulated results from carbohydrate fermentations among these strains are:

[42] J. Lab. and Clin. Med., 1919, 4, p. 6.

TABLE 5

RESULTS FROM CARBOHYDRATE FERMENTATIONS

Percentage Fermentations in	Typhoid Strains (48 Strains)	Strains from an Individual after Administration of Purgative (20 Strains)	Strains from Persons with Nonspecific Diarrheas (2 Subjects; 10 Strains from Each)
Glucose	100	90	100
Lactose	95	80	100
Salicin	90	90	90
Inulin	0	0	0
Mannite	25	50	25

The foregoing data are not strictly comparable to those of the studies made on the fecal streptococci of normal stools, since they represent a much smaller aggregate of observations. It is, however, highly suggestive. It was noted in all cases that fermentation of glucose was much slower than in the case of the streptococci from normal stools, usually requiring three times as long to be complete. In general, all fermentative activity seemed to be retarded or inhibited to some extent in this series. Whether this explains in part the fact that Fuller and Armstrong as well as myself obtained a higher percentage of fermentations than Winslow and Palmer, who used, for the most part, strains from diarrheal material for study, will require a more extensive series of observations to decide.

If further work were to reveal that fermentative characteristics of the fecal streptococci vary from the norm in diarrheal material, three questions will be raised, namely:

1. Are such variants, failing to ferment mannite, identical with the nonmannite fermenters normally found in the buccal cavity and nasopharynx, and not converted into more active fermentative forms due to unnatural conditions in the alimentary tract or unduly rapid transit through it?

2. Are these less active fermenters less resistant and less active forms normally found in the intestinal tract, but not hardly enough under normal conditions to remain viable in as great numbers until passed with the feces?

3. Or are they the characteristic mannite fermenting organism, active fermentatively, but rather permanently impaired in fermentative characters by marked environmental changes? These questions remain as interesting topics for further study, should the evidence furnished by these 84 strains prove true in the case of a larger number. A matter of some interest is the apparent preponderance of streptococci over other organisms on blood-agar plates from diarrheal stools. The proportionate number is perceptibly greater than in the case of nondiarrheal stools.

Eight stools from 3 healthy infants were examined, and streptococci recovered from 5 stools. The youngest subject was not quite 1 month old, and 3 examinations were necessary before suitable plates could be obtained. The next youngest was about 6 months of age, and plates from 2 out of 3 samples showed streptococci. The oldest subject was approximately 2 years of age, and yielded plates showing streptococci in the first 2 samples examined. It would appear from the number of streptococci found on the plates that their numerical frequency in infants is not so great as in the feces of adults.

Altogether, but 34 strains from infants were studied. They presented no striking differences from those found in the feces of adults with regard to

morphology or appearance of colonies on the blood-plates. In fermentative reaction on carbohydrates, they presented the following gross percentages:

Glucose	100
Lactose	100
Salicin	97
Inulin	0
Mannite	82

The foregoing number is probably insufficient to use in comparison with the 323 strains reported from normal adult feces; it is, however, demonstrative of the fact that the fermentative types commonly found in adult feces are established at a very early age, and are found normally even in very young infants.

SUMMARY

Frequent allusions have been made in the literature to the streptococcus as an etiologic factor in the diseases of the alimentary tract. The lack of means of sharp demarcation between the streptococcus as a normal inhabitant of the bowel and as an incitor of specific disease, and the position of the streptococcus as a common secondary invader, make it difficult to confirm the clinical evidence offered.

Mannite fermenting nonhemolytic streptococci are the characteristic predominant types found in the feces of normal individuals.

Mannite fermentation is quantitatively less uniform, among the strains of human fecal streptococci, than that of other carbohydrates; it is, however, the most important fermentative characteristic, its numerical frequency depending to a great extent on the method of cultivation employed.

The "indifferent" variety of streptococcus in the feces of normal persons is inconstant in its indifference to the formation of green; green-formation would seem to be favorably influenced by the alkalinity of the mediums.

Hemolytic streptococci are but exceptional findings in the stools of normal, healthy people, and are inconstant in occurrence.

No apparent morphologic characteristics aside from those dependent on rapid, luxurious growth, characterize the human fecal streptococci.

Examination of the diarrheal stools from specific and nonspecific diarrheas as well as after catharsis, would suggest the presence of streptococci not so marked fermenters of mannite, and less active generally in the fermentation of carbohydrates.

Stools from infants contain streptococci, slightly less in numerical proportion than those of adults, but conforming in morphologic and fermentative types quite closely with those found in the feces of adults.